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Mate choice and the operational sex ratio: an experimental test with robotic crabs

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Abstract: The operational sex ratio (OSR: sexually active males: receptive females) predicts the intensity of competition for mates. It is less clear, however, under what circumstances, the OSR predicts the strength of sexual selection – that is, the extent to which variation in mating success is attributable to traits that increase the bearer's attractiveness and/or fighting ability. To establish causality, experiments that manipulate the OSR are required. Furthermore, if it is possible to control for any OSR-dependent changes in the chosen sex (e.g. changes in male courtship), we can directly test whether the OSR affects the behaviour of the choosing sex (e.g. female choice decisions). We conducted female mate choice experiments in the field using robotic models of male fiddler crabs (*Uca mjoebergi*). We used a novel design with two females tested sequentially per trial. As in nature, the choice of the first female to mate therefore affected the mates available to the next female. In general, we detected significant sexual selection due to female choice for 'males' with larger claws. Importantly, the strength of sexual selection did not vary across five different OSR/density treatments. However, as the OSR decreased (hence the number of available males declined), females chose the 'males' with the largest claws available significantly more often than expected by chance. Possible reasons for this mismatch between the expected and observed effects of the OSR on the strength of sexual selection are discussed.

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10 **Mate choice and the operational sex ratio: an experimental**
11 **test with robotic crabs**

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37 **Title:** Mate choice and the operational sex ratio: an experimental test

38 **Abbreviated title:** Mate choice and the OSR
39
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Abstract: The operational sex ratio (OSR) (sexually active males: receptive females) predicts the intensity of competition for mates. It is less clear, however, under what circumstances the OSR predicts the strength of sexual selection – that is, the extent to which variation in mating success is attributable to traits that increase the bearer’s attractiveness and/or fighting ability. To establish causality experiments are required that manipulate OSR. Furthermore, if it is possible to control for any OSR-dependent changes in the chosen sex (e.g. changes in male courtship), we can directly test whether the OSR affects the behaviour of the choosing sex (e.g. female choice decisions). We conducted female mate choice experiments in the field using robotic models of male fiddler crabs (*Uca mjoebergi*). We used a novel design with two females tested sequentially per trial. As in nature, the choice of the first female to mate therefore affected the mates available to the next female. In general we detected significant sexual selection due to female choice for ‘males’ with larger claws. Importantly, the strength of sexual selection did not vary across five different OSR/density treatments. However, as the OSR decreased (hence the number of available males declined), females chose the ‘males’ with the largest claws available significantly more often than expected by chance. Possible reasons for this mismatch between the expected and observed effects of the OSR on the strength of sexual selection are discussed.

Keywords: density, female choice, mate sampling, mating preferences

INTRODUCTION

It is well established that sexual selection has led to the evolution of costly male traits that are advantageous during male-male contests, or when competing to attract females (Andersson, 1994). It is less clear what demographic or ecological factors generate variation among species or populations in these secondary sexual traits (Cornwallis & Uller, 2010). One parameter traditionally used to predict the strength of sexual selection is the operational sex ratio (OSR: ratio of sexually active males to receptive females) (Emlen & Oring, 1977; for a review of sexual selection indices see Henshaw *et al.*, 2016). By definition the intensity of competition for mates is stronger when the OSR is more biased. Greater mating competition need not, however, equate to stronger sexual selection. This is because sexual selection is measured as the *response* to competition (i.e. the extent to which variation in mating success among individuals is due to differences in the expression of sexual traits) (Shuster, 2009; Klug *et al.*, 2010; Jennions *et al.*, 2012). In some cases, it is more profitable to invest in other fitness-enhancing traits when the intensity of mating competition increases (e.g. parental care; Kokko & Jennions, 2008). As the OSR becomes increasingly male-biased, variation in male mating success attributed to individual differences in sexually selected traits might therefore even decrease.

There are several reasons why the relationship between the OSR and the strength of sexual selection on focal traits cannot be derived from first principles. First, there is an imperfect correlation between the intensity of sexual competition (i.e. OSR) and a sexually selected response (e.g. greater investment in ornaments or weaponry) if there are also ways to increase fitness via naturally selected traits (e.g. parental care,

defence against parasitism) (reviews: Kokko & Jennions, 2008; Jones, 2009). Second, proximate factors that affect the intensity of sexual selection on different traits can change with the OSR (Shuster, 2009; Klug *et al.*, 2010). For example, controlling for density, when the OSR is more male-biased the encounter rates between males will increase. Numerous aggressive interactions might make harem defence less economically viable in some species (Emlen & Oring, 1977; Klemme *et al.*, 2007). This could reduce sexual selection on weapons, but might increase sexual selection on other sexually selected traits (e.g. testes size if females are then more inclined to mate multiply). Third, although it is counter-intuitive, all else being equal, female mate sampling tactics that involve assessment of a random subset of males yield the same strength of sexual selection on preferred male traits regardless of the OSR (Klug *et al.*, 2010). Fourth, all else is not necessarily equal, however, as greater choice can reduce the ability of choosy individuals to identify preferred items, including mates (e.g. consumers/shoppers: Lenton & Francesconi, 2011; but see Scheibehenne *et al.*, 2010; mate-searching animals: Hutchinson, 2005; Alem *et al.*, 2015). The density of one sex and the OSR are related, and they covary perfectly if the population density is constant. Consequently, with a more biased OSR, there are often more mates available for the rarer sex to choose from at any moment.

There is no formal theoretical link between the OSR and the strength of sexual selection on focal male traits, so the true relationship has to be determined empirically (Klug *et al.*, 2010). Comparative analyses suggest that male-biased sex ratios are generally associated with greater male ornamentation and/or weaponry, but the OSR covaries with other factors, notably the adult sex ratio, so it is problematic to assign a causal role to the OSR (Fromhage & Jennions, submitted). Experimental studies that

manipulate the OSR to determine its causal effects yield contrasting results. For example, as expected, sexual selection on male body size increased as the OSR was made more male-biased in two-spotted gobies, *Gobiusculus flavescens* (Wacker *et al.*, 2013). There was, however, no relationship between the OSR and the strength of sexual selection on attractive male traits in guppies, *Poecilia reticulata* (Head *et al.*, 2008) or mosquitofish, *Gambusia holbrooki* (Head *et al.* submitted); and there was weaker sexual selection on male body size in bank voles *Clethrionomys glareolus* (Klemme *et al.*, 2007) and common lizards *Lacerta vivipara* (Fitze & Le Galliard, 2008) when the OSR was made more male-biased.

Experiments manipulating the OSR are needed to determine whether general trends can be identified. For example, is the OSR a better predictor of sexual selection in some types of mating systems and/or taxa than others? Such OSR manipulation experiments, while straightforward, are surprisingly few in number. Even more rare are studies where OSR-dependent behavioural changes in one sex are controlled for, so that the direct effect of the OSR on the other sex can be calculated. For example, if female choice based on male courtship varies with the OSR, is this due to OSR-dependent changes in how males court, or in how females evaluate males? To tease sex-specific effects apart necessitates experiments that use artificial stimuli, such as acoustic playbacks or robots, so that ‘male’ behaviour is unaffected by the OSR.

Here, we examine how the OSR affects female choice for male claw size in fiddler crabs. We tested females with custom-built robots ‘males’ that we have successfully used in many previous studies (e.g. Reaney, 2009; Kahn *et al.*, 2013). To avoid any confusion about our non-standard experimental design (two females per trial, see

Methods), we note three points. First, the OSR changes constantly but, to be a useful predictive tool, it should be measured at a biologically appropriate scale. For example, 100 calling male frogs and 20 gravid females might be at a pond over a night. Most researchers would report this as a 5:1 OSR (e.g. Ryan, 1981). Strictly speaking, however, for the first female the OSR was 100:1 and for the last female it was 81:1 (male frogs rarely return to the mating pool on the night that they mate). In general, most researchers are interested in this type of ‘population level’ OSR. Unless otherwise stated we follow this convention in our study. Specifically, we refer to the OSR at the start of an experimental trial rather than that experienced by successive females within a trial. It is less common to refer to the OSR experienced by an individual female. This is probably because the ‘female-level’ OSR is synonymous with how many sexually receptive males a female encounters. In such case, rather than refer to the OSR, we simply refer to the number of males available as mates.

Second, most mate choice experiments test a single female per scenario so that each female chooses from the same set of stimuli. The disadvantage, hinted at in the frog scenario, is that this design ignores changes in mate availability that will arise when females sequentially arrive to mate. Biologically realistic mate choice experiments could include the possibility that several females chose from the same set of males but that earlier choices constrain future options. Third, as noted earlier, the population level OSR and the total number of available males (‘male density’) tend to be correlated in the field. Although density and the population level OSR can be teased apart in experiments (Kokko & Rankin, 2006; Wacker *et al.*, 2013), this does not negate the fact that a more male-biased population level OSR can often equally well be described as a case where females choose among a greater number of males.

Researchers interchangeably describe this as the effect of either greater choice of mates (standard terminology for those interested in proximate sensory mechanisms) or of the OSR (standard terminology for those interested in how sex ratios affect selection gradients) on female mating preferences.

METHODS

We studied *Uca mjoebergi* at East Point Reserve in Darwin, Australia in October-December 2010. Both sexes defend burrows on inter-tidal mudflats. During the five-day mating period that occurs each semi-lunar tidal cycle, mate-searching females leave their burrow, move across the mudflat and sequentially encounter clusters of 2-10 males. This is the main level at which mate choice decisions occur. The population level OSR is always male-biased. Males court females by vigorously waving their enlarged major claw. In the field, females more often approach larger-clawed males (Reaney & Backwell, 2007) and males that wave earlier (i.e. produce ‘leading’ waves) (Reaney *et al.*, 2008). After choosing a male the female enters his burrow to inspect it. Burrow features then influence whether she stays and breeds, or continues mate searching (Reaney & Backwell, 2007). Sexual selection presumably favours males with large claws that increase the likelihood that a female inspects his burrow. Male claw size in the population at the time of our study ranged from 4.6 to 25.4 mm (mean \pm SD: 14.97 \pm 4.29 mm; $n = 222$; based on transects). This is consistent with three recent estimates from the same study area [14.84 mm ($n = 82$), 14.95 mm ($n = 199$) and 14.9 mm ($n = 177$); Clark & Backwell 2015). Even though males within this size range all produce courtship waves, it is possible that the smaller males have burrows that are too narrow for females. A better estimate of the size range of males

available as mates is obtained by restricting the dataset to males whose burrows are inspected by females. Three recent estimates from the field at the same site for the mean \pm SD claw size of these males are 14.84 ± 1.63 ($n = 37$), 16.05 ± 2.29 ($n = 135$) and 16.60 ± 2.92 mm ($n = 57$) (data from Clark & Backwell 2015).

We ran mate choice experiments where we sequentially presented two test females with 3, 5 or 7 robotic ‘males’ (i.e. the population level OSR over the trial was 7:2, 5:2 or 3:2, and the OSR for individual females ranged from 7:1 to 2:1). Each robot consists of a painted cast of a claw attached to a motorised metal arm that mimics courtship waving (details in Reaney *et al.*, 2008). Robots waved in synchrony so that leadership did not affect female choice. The test arena was an area of mudflat from which we removed all resident crabs. We used mate-sampling females caught *in situ* who were measured (carapace width ± 0.1 mm) and held in individual containers until tested (<30 mins). We placed the first female under a clear plastic cup at a point equidistant from all robots (20 cm). After the female settled we raised the cup and scored a choice decision if she moved to <2 cm of a ‘male’. A female was discarded and replaced with another if she ran immediately after release, or did not choose within 3 minutes ($n = 47$). This criterion for choice produces highly repeatable results in the many studies we have conducted (e.g. Reaney, 2009; Kahn *et al.*, 2013). The results from female choice trials using robotic crabs have largely been corroborated by field studies of correlates of male mating success (e.g. Reaney & Backwell, 2007; Clark & Backwell 2015). Given the large numbers of mate-searching females, and that females mate on the day that they begin to search (Clark & Backwell 2015), it is highly improbable that we tested a female more than once. The first female saw all the ‘males’ wave before she was released. Her chosen ‘male’ was then removed and the

214 second female chose from the remaining ‘males’. This mimics the natural situation. In
215 thousands of hours of field observations we have almost never seen two females
216 simultaneously approach a cluster of males – rather, females sequentially approach
217 sets of males. Mate choice by an earlier female removes the chosen male from the set
218 of potential mates of the next female because a male remains underground once a
219 female has chosen to stay in his burrow. Ideally we would have collected data blind to
220 the OSR and male claw size, but this was not possible (see Holman *et al.* 2015).
221 Female choice of a given ‘male’ is, however, clear-cut based on her behaviour, and
222 we had no *a priori* expectations as to how the OSR would affect female choice.
223
224 We used three high-density treatments where the spacing between ‘males’ was
225 constant (5 cm) to test if the OSR affects the strength of sexual selection: 7:2, 5:2 and
226 3:2 (Fig 1a). Our design reflects the clusters of waving males encountered by a mate-
227 searching female. The smallest, median and largest claw sizes we used were always
228 the same (Table 1). The mean ‘male’ claw size (18.2 mm) was larger than the mean
229 for males whose burrows are inspected (16.0 mm; Clark & Backwell, 2015): 10 of
230 the 15 ‘males’ had claws greater than 16.0 mm. This makes tests for a directional
231 mating preference conservative, although only moderately so given that naturally 7 of
232 15 males are larger than average (assuming the median and the mean are the same).
233 The chosen size range was necessary to ensure the tested claws spanned the natural
234 size range, but were evenly distributed in size while keeping the mean value constant.
235 The position of robots with different sized claws was randomised. To test for an
236 effect of male density independent of the OSR (e.g. Head *et al.*, 2008, Wacker *et al.*,
237 2013) we re-ran the 5:2 and 3:2 OSR treatments at lower densities, adjusting the
238 spacing between robots to cover the same area as the 7:2 treatment (Fig. 1b). We ran

40 trials (= 80 females) per treatment ($n = 400$ choice trials). Each female was used in one trial/treatment. We randomised the order of the treatments during each day of testing.

To quantify sexual selection we calculated the selection differential (s) as the mean size of the chosen claws minus the original mean claw size. Unless otherwise stated this is based on the mean value of the two females. We ran t -tests to determine whether s differed from zero, and an ANOVA to test whether s differed among the OSR treatments with even ‘male’ spacing. We used a general linear model to test whether density (high/low) and its interaction with the OSR (3:2 or 5:2) affected s . There was no effect of day of cycle (relative to spring tide) on s (or the difference between the observed and maximum possible value of s), nor did day of cycle interact with OSR treatment (all $p > 0.254$).

When the OSR is more male-biased, the mean size of the two largest males is greater (Table 1). To determine whether female choice is more error prone when the OSR is more male-biased it is tempting to test whether the difference between the mean size of the chosen males and the maximum mean possible increases with the OSR. This is, however, a problematic approach as the probability that by chance alone the two largest males are chosen is higher when the OSR is less male biased (i.e. there are fewer males). The null prediction is that, by chance, the two largest males are chosen 1 in 3 times when the OSR is 3:2, 1 in 10 times when the OSR is 5:2 and 1 in 21 times when the OSR is 7:2. We therefore tested whether the observed number of times the two largest ‘males’ were chosen was significantly greater than expected using separate one-tailed binomial tests. To indirectly compare female error rates among the

high density OSR treatments we ran an ANOVA to test whether the claw size chosen by the first female per trial differed (this is equivalent to asking whether s differed because the mean claw size is identical for all three OSRs).

Finally, we compared s between the first and second female (s for the second female was based on the mean size of the remaining available claws) with a paired t -test. We tested whether there was an effect of the OSR treatment on the difference in s between the first and second female using separate ANOVAs for high and low density tests. We also calculated the correlation between female size and chosen claw size for each treatment. We used the first female per trial to ensure comparable male availability. All tests were two-tailed ($\alpha = 0.05$) and run in SPSS 19.0.

RESULTS

In the high-density treatments the selection differential was significantly greater than zero at all three OSRs. Females prefer larger claws: 7:2 ($t_{39} = 2.968, p = 0.005$), 5:2 ($t_{39} = 2.507, p = 0.016$) and 3:2 ($t_{39} = 3.798, p < 0.001$) (Table 1). The strength of sexual selection (s) did not depend on the OSR ($F_{2,117} = 0.242, p = 0.785$; 7:2 = 1.187mm; 5:2 = 0.98mm ; 3:2 = 1.35mm). Females in the 3:2 treatment more often chose the two largest available claws than did females in the 5:2 or 7:2 treatments. The frequency of trials in which the two largest males were chosen was significantly greater than expected by chance for the 3:2 treatment (24 of 40 trials, $p = 0.001$), but not for the 5:2 (4 of 40 trials) or 7:2 treatments (2 of 40 trials) (both $p > 0.50$). There was, however, no evidence that the first female more often selected the largest claw at a less male-biased OSR as the mean claw size in first trials did not differ across

treatments ($F_{2,117} = 0.482$, $p = 0.62$; mean claw size: 3:2 = 18.35mm,; 5:2 = 19.08mm, 7:2 = 19.19 mm). The greater than expected number of choices of the two largest males in the 3:2 treatment must therefore be driven by the choice of the second female.

In the low-density treatments, although females still tended to choose larger claws, sexual selection for larger claws was not significant at either a 3:2 ($t_{39} = 1.312$, $p = 0.197$) or 5:2 OSR ($t_{39} = 1.936$, $p = 0.06$). The frequency of trials in which the two largest males were chosen was, however, not significantly greater than expected by chance in either the 3:2 treatment (18 of 40 trials, $p = 0.083$), or the 5:2 treatment (7 of 40 trials, $p = 0.10$). There was no evidence that the first female more often selected the largest claw at a less male-biased OSR, as the mean claw did not differ ($F_{1,78} = 0.860$, $p = 0.357$; 3:2 = 19.55mm,; 5:2 = 18.55mm).

When analysing results from the two densities together, there was no significant effect of density on the strength of sexual selection (s) ($F_{1,156} = 2.076$, $p = 0.152$), nor was there a density by OSR interaction ($F_{1,156} = 0.548$, $p = 0.460$) or a main effect of OSR ($F_{1,156} = 0.063$, $p = 0.802$) (Mean s was 1.35 and 0.525 mm at high and low densities for the 3:2 OSR, and 0.975 and 0.710 mm at high and low densities for the 5:2 OSR).

Combining all available trials there was no evidence that the selection differential of the second female was stronger than that of the first female ($t_{199} = 1.533$, $p = 0.127$; first = 0.756 mm, second = 1.389 mm). There was no effect of the OSR on the magnitude of the difference in the selection differential between the first and second female (low-density: $F_{2,117} = 1.779$, $p = 0.173$; high-density: $F_{1,78} = 1.653$, $p = 0.202$;

the direction of the effect was first $s <$ second s at both densities).

Finally, female size was uncorrelated with chosen claw size in all five treatments ($r = 0.173, -0.058, 0.157, 0.053, -0.027$, all $p > 0.285$). The mean correlation was $r = 0.060$ ($t_4 = 1.275, p = 0.271$). There was no evidence that larger claws additionally enhance male fitness by preferentially attracting larger, more fecund, females.

DISCUSSION

Female *Uca mjoebergi* preferred to visit larger-clawed robotic ‘males’ in three of our five OSR/density treatments, and there was a marginally non-significant preference in one treatment ($p = 0.06$). These results corroborate findings from comparable mate choice experiments with robotic crabs (e.g. Reaney, 2009; Kahn *et al.*, 2013). They also agree with our estimates of male mating success in the field (Reaney & Backwell, 2007). Our mate choice experiments were conservative with respect to female choice for larger males as the mean test claw size (18.2mm) was larger than the population mean (14.9 mm). The disparity was smaller when using the estimated mean of 16.0mm based on males naturally visited by females (from Clark & Backwell 2015). Ten of the 15 ‘males’ available across the three OSR treatments were larger than this mean. The strong directional female mating preference for larger claws that we still observed can therefore partly explain why, like all fiddler crabs, male *U. mjoebergi* have a greatly enlarged major claw.

Although directional selection on claw size was not statistically significant in all treatments, the strength of sexual selection due to female choice did not depend on the

OSR. More specifically, sexual selection was not stronger when the OSR was more male-biased. In general, this positive trend is widely predicted because the mean size of the two largest claws was *bigger* when the OSR was more male biased (Table 1). If each female chose the largest available claw with the same propensity irrespective of the OSR, the strength of sexual selection should have *increased* with a more male-biased OSR. (There is no confounding effect of mate-sampling tactics affecting the mean size of available males [for examples see Klug *et al.*, 2010] because females had simultaneous access to all available ‘males’). Instead, we found that the females’ propensity to choose the two largest claws available decreased as the OSR became more male-biased. Specifically, females chose the two largest claws significantly more often than expected by chance only in the least male-biased OSR (3:2, at high density). The absence of a relationship between the OSR and strength of sexual selection in *U. mjoebergi* is a reminder that the OSR is an imperfect predictor (Jones, 2009; Shuster, 2009). Indeed, a recent simulation study of various mating systems that tested a range of proposed indices of sexual selection, showed that the OSR tended to be a consistently poor predictor of sexual selection on a focal trait (Henshaw *et al.*, 2016).

There are several possible reasons why the OSR did not predict the strength of sexual selection driven by female choice for larger claws in *U. mjoebergi*. To start though, we can eliminate any role of OSR-dependent changes in male behaviour (review: Weir *et al.*, 2011) that might affect the attractiveness of a larger claw to females. This is because we tested females using robotic ‘males’ with constant wave rates. An OSR-mediated change in non-focal traits is potentially important in the field because the correlation between claw size and wave rate, which are both traits affecting male

attractiveness, varies over the mating period in *U. mjoebergi* (Kahn *et al.*, 2013).

We suggest that the OSR affected the strength of sexual selection due to psycho-sensory processes that altered a female's ability to discriminate between males and chose the largest available. The greater potential for stronger sexual selection with a more male-biased OSR (because the two largest available claws have a greater mean size) is then negated by increased mistakes by females so that there is no net effect of the OSR on the strength of sexual selection. There are two factors likely to increase female error rates in identifying the largest claw. First, the size difference between adjacent sized 'males' declined from 6 mm to 2 mm as the OSR went from 3:2 to 7:2 (Table 1). This is not an experimental flaw: it is inevitable that the size difference between adjacent sized 'males' decreases when more are present. Discrimination between similar sized objects is always more difficult (citations in Abbas *et al.*, 2013). It is worth noting that the mean claw size chosen by the first female did not differ among the OSR treatments. However, the proportion of tests in which the two largest males were selected was greater than expected by chance was only significantly for the 3:2 OSR treatment. This suggests that it is the choice of the second female that drives this result, implying that when there are only two males to chose between in the 3:2 OSR treatment females are more likely to select the largest available male than when there are four or six available males as in the 5:2 and 7:2 OSR treatments.

Second, the number of 'males' present increased with the OSR, again reflecting the situation in the field. There is some evidence that choice of a preferred item becomes more difficult when there are more items to choose from (review: Hutchinson, 2005). Together this implies that the number of 'males' and/or size-differences between

‘males’ that both covaried with the OSR increased the error rate of choosy females. Similar relationships seem likely to occur in many taxa where the biologically relevant OSR largely depends on the number of males present on a breeding/mating site while individual females are sampling males. Arguing against a role for the number of males and/or size-differences between males is that there was no significant increase in the selection differential of the second female relative to that of the first female. This occurred even though the second female saw one fewer ‘male’, and, on average, there was a greater size difference between the largest and next largest ‘male’. It is worth noting, however, that we might have reported a significant effect if we had used a different distribution of claw sizes so that there were fewer ‘males’ with above average sized claws (relative to the natural mean). In such a case, the choice of a large ‘male’ by the first female would have increased the proportion of below average ‘males’ so that females might have become less choosy. More convincingly, in previous two-choice experiments the only treatment in which females did *not* choose the bigger claw was when they differed in size by just 2 mm (Reaney 2009).

To test whether the number of ‘males’ *per se* has a direct effect on female choice it is necessary first to control for the size differences between ‘males’, *and* vary the number of ‘males’ (e.g. double the number of ‘males’ in the 3:2 OSR treatment and compare the 3:2 and 6:2 treatments). It should be noted, however, that in the proposed, as well as the current, experiment the variance in male claw size is greater when the OSR is less male biased. This is a constraint of the design given the decision to have the same range in claw size. In a natural setting the number of males that an individual female encounters might not show the same relationship with variation in

male claw size. Finally, it is possible that the OSR directly changes female mating preferences. Females might have a weaker preference for larger males when, say, more males are present although there is no obvious adaptive advantage to such a shift in mating preference.

In conclusion, the proximate mechanisms underlying consistent sexual selection for larger claw size independent of the OSR in *U. mjoebergi* are unknown. This does not, however, negate our key finding sexual selection theory. Despite the OSR covarying with the opportunity for sexual selection (i.e. maximum selection differential) sexual selection on male claw size did not increase as the OSR became more male-biased.

DATA ACCESSIBILITY

Data for this study is available from the DRYAD repository doi:10.5061/dryad.5qb78

REFERENCES

- Abbas, S.S., Dijkstra, T.M.H. & Heskes, T. 2013. A direct comparison of visual discrimination of shape and size on a large range of aspect ratios. *Vision Research* **91**: 84-92.
- Alem, S., Clanet, C., Party, V., Dixsaut, A. & Greenfield, M.D. 2015. What determines lek size? Cognitive constraints and per capita attraction of females limit male aggregation in an acoustic moth. *Anim. Behav.* **100**: 106-115.
- Andersson, M.B. 1994. *Sexual Selection*. Princeton University Press, Princeton, NJ.
- Clark, H.L. & Backwell, P.R.Y. 2015. Temporal and spatial variation in female mating preferences in a fiddler crab. *Behav. Ecol. Sociobiol.* **69**: 1779–1784.

439 Cornwallis, C.K. & Uller, T. 2010. Towards an evolutionary ecology of sexual traits.
440 *Trends Ecol. Evol.* **25**: 145-152.

441 Emlen, S.T. & Oring, L.W. 1977. Ecology, sexual selection, and the evolution of
442 mating systems. *Science* **197**: 215-223.

443 Fitze, P.S. & Le Galliard, J.F. 2008. Operational sex ratio, sexual conflict and the
444 intensity of sexual selection. *Ecol. Lett.* **11**: 432-439.

445 Head, M.L., Lindholm, A.K. & Brooks, R. 2008. Operational sex ratio and density do
446 not affect directional selection on male sexual ornaments and behavior. *Evolution*
447 **62**: 135-144.

448 Henshaw, J., Kahn, A.T. & Fritzsche, K. 2016. A rigorous comparison of sexual
449 selection indices via simulations of diverse mating systems. *Proc Natl. Acad. Sci.*
450 *U.S.A.* **113**: E300-E308.

451 Holman, L., Head, M.L., Lanfear, R. & Jennions, M.D. 2015. Evidence of
452 experimental bias in the life sciences: why we need blind data recording. *PLoS*
453 *Biology* **13**: e1002190.

454 Hutchinson, J.M.C. 2005. Is more choice always desirable? Evidence and arguments
455 from leks, food selection, and environmental enrichment. *Biol. Rev.* **80**: 73-92.

456 Jennions, M.D., Kokko, H. & Klug, H. 2012. The opportunity to be misled in studies
457 of sexual selection. *J. Evol. Biol.* **25**: 591-598.

458 Jones, A.G. 2009. On the opportunity for sexual selection, the Bateman gradient and
459 the maximum intensity of sexual selection. *Evolution* **63**: 1673-1684.

460 Kahn, A.T., Dolstra, T., Jennions, M.D. & Backwell, P.R.Y. 2013. Strategic male
461 courtship effort varies in concert with adaptive shifts in female mating
462 preferences. *Behav. Ecol.* **24**: 906-913

463 Klemme, I., Ylonen, H. & Eccard, J.A. 2007. Reproductive success of male bank

464 voles (*Clethrionomys glareolus*): the effect of operational sex ratio and body size.
 465 *Behav. Ecol. Sociobiol.* **61**: 1911-1918.

466 Klug, H., Heuschele, J., Jennions, M.D. & Kokko, H. 2010. The mismeasurement of
 467 sexual selection. *J. Evol. Biol.* **23**: 447-462.

468 Kokko, H. & Jennions, M.D. 2008. Parental investment, sexual selection and sex
 469 ratios. *J. Evol. Biol.* **21**: 919-948.

470 Kokko, H. & Rankin, D.J. 2006. Lonely hearts or sex in the city? Density-dependent
 471 effects in mating systems. *Phil. Trans. Roy. Soc. B.* **361**: 319-334.

472 Lenton, A.P. & Francesconi, M. 2011. Too much of a good thing? Variety is
 473 confusing in mate choice. *Biol. Letts.* **7**: 528-531.

474 Reaney, L.T. & Backwell, P.R.Y. 2007. Temporal constraints and female preference
 475 for burrow width in the fiddler crab, *Uca mjoebergi*. *Behav. Ecol. Sociobiol.* **61**:
 476 1515-1521.

477 Reaney, L.T., Sims, R.A., Sims, S.W., Jennions, M.D. & Backwell, P.R.Y. 2008.
 478 Experiments with robots explain synchronized courtship in fiddler crabs. *Curr.*
 479 *Biol.* **18**: R62-R63.

480 Reaney, L.T. 2009. Female preference for male phenotypic traits in a fiddler crab: do
 481 females use absolute or comparative evaluation? *Anim. Behav.* **77**: 139-143.

482 Ryan, M.J. 1981. *The Tungara Frog*. University of Chicago Press, Chicago.

483 Scheibehenne, B., Greifender, R. & Todd, P.M. 2010. Can there ever be too many
 484 options? A meta-analytic review of choice overload. *J Consumer Research* **37**:
 485 409-425.

486 Shuster, S.M. 2009. Sexual selection and mating systems. *Proc. Natl. Acad. Sci.*
 487 *U.S.A.* **106**: 10009-10016.

488 Wacker, S., Mobley, K., Forsgren, E., Myhre, L.C., de Jong, K. & Amundsen, T.

2013. Operational sex ratio but not density affects sexual selection in a fish.

Evolution **67**: 1937-1949.

Weir, L.K., Grant, J.W.A. & Hutchings, J.A. 2011. The influence of operational sex ratio on the intensity of competition for mates. *Amer. Nat.* **177**: 167-176.

Table 1. Available, observed mean of chosen males, and maximum possible mean claw length (i.e. largest two males) (in mm) for the five OSR-density treatments.

Treatment			Chosen mean \pm SD (all $n=40$ trials)		Maximum possible mean
OSR	Male claw length	Mean	High density	Low density	
7:2	12.2, 14.1, 16.2, 18.2, 20.2, 22.1, 24.2	18.2	19.34 \pm 2.53		23.15
5:2	12.2, 15.2, 18.2, 21.1, 24.2	18.2	19.16 \pm 2.46	18.89 \pm 2.32	22.65
3:2	12.2, 18.2, 24.2	18.2	19.55 \pm 2.25	18.73 \pm 2.53	21.2

Figure 1. Diagram of the three OSR treatments: (a) even male spacing (high density) and (b) spacing such that the same area is covered in all three OSR treatments. Claws: robotic ‘males’; crab: female release point.

